



Dedicated to the genetic improvement of U.S. rosaceous crops

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Volume 2 Issue 1

RosBREED's Second Annual Meeting with Advisory Panel Members, San Diego, CA January

RosBREED met in San Diego, California for the RosBREED II Annual Meeting with its Industry, Scientific, and Extension Advisory Panel Members on January 13, 2011. Thirty-nine RosBREED participants including 21 Advisory Panel Members were present at the meeting, which focused on project outcomes, impacts, and challenges faced to date as well as our second year planned outcomes and impacts. Face-to-face discussion sessions were a highlight, emphasizing input from the panels. RosBREED Teams interacting directly with Advisory Panels obtained much valuable feedback for future success.

At the conclusion of the day, each Advisory Panel Member was asked to describe one positive and one negative about what they had seen and heard. This is what Phil Korson (President, Cherry Marketing Inst., Inc., Lansing, MI) had to say "I am happy to be serving as an Advisory Panel Member for a great program like RosBREED and having this opportunity to come together and meet with other Industry Advisory Panel Members. We all e-mail each other but it's great to converse face to face."



Chengyan Yue, Socio-Economics Team Leader, and Phil Korson, member of RosBREED's Industry Advisory Panel



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Photo: Jim Hancock

Diploid Strawberry Has Been Sequenced

The genome sequence of the woodland strawberry, *Fragaria vesca*, (diploid and a much less complex strawberry compared to the cultivated octoploid species, *Fragaria x ananassa*) was published in December, 2010. Within the 240 million base pairs sequenced, genes believed to be conferring fruit quality traits such as flavor, aroma and nutritional value were identified among others, which is exciting news for RosBREED! Other than *Arabidopsis*, woodland strawberry as well as peach, another member of the Rosaceae family, are two of the smallest plant genomes thus far sequenced.

For more information, please visit:

<http://www.nature.com/ng/journal/vaop/ncurrent/full/ng.740.html> or
<http://www.strawberrygenome.org/>



Project Co-PDs and Project Associates: RosBREED's Annual Scientific Meeting will be held in East Lansing, MI, on March 8-9, 2011, followed by two and a half days of training in Pedigree-Based Analysis for crop breeding teams (March 9-11). A fruit show-and-tell-and-taste session on the first day's afternoon break will focus on traits of currently available fruit selections and what is on the horizon!

RosBREED by the numbers

1330 KiloBytes

Size of the data file of fruit quality and other trait evaluation data obtained by the University of Minnesota apple breeding program at harvest. Traits include fruit shape, skin color, sensory data as well as tree diseases and disorders. 255 trees of cultivars, selections, and seedlings representing breeding parents important to the Minnesota program were evaluated with standardized phenotyping protocols established pre-season and [available online to all breeders and researchers](#). The harvest season lasted for nearly 9 weeks and phenotyping was completed by 2 Breeding Trainees (Matt Clark and Cari Schmitz), 1 undergraduate student, and 1 steadfast volunteer.



Cari Schmitz and Matt Clark, RosBREED Breeding Trainees, Univ. of Minnesota

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Number of promising trait-predictive markers (for fruit size, firmness, sweetness, and acidity) that the Genotyping Team screened on the tart and sweet cherry reference germplasm (cherry Crop Reference Set) of 500 trees. The three markers are associated with an important genetic region for fruit size in cherry, and are also helpful for pedigree confirmation. In coming months, the ability of the markers to predict superior fruit quality will be determined with FlexQTL™. If validated, genotype-based breeding values of breeding parents will be calculated and used in crossing decisions!



April Nyberg, USDA-ARS-NCGR

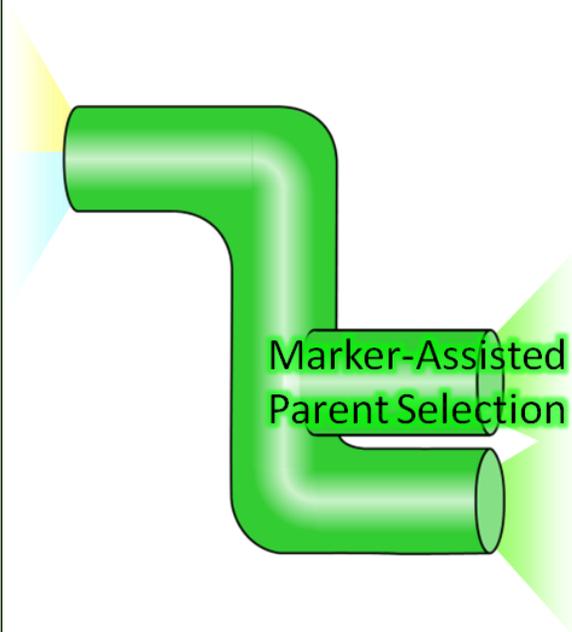
Community Breeders' Page

The Marker-Assisted Breeding Pipeline: Channeling socio-economic and DNA information into routine breeding operations

By Cameron Peace, MAB Pipeline Team Leader

In this issue:

Informing crossing decisions in Rosaceae crop breeding



Crossing is arguably the most influential, far-reaching decision that Rosaceae crop breeders can make. The breeder has in mind the next generation – thousands of plants that must be cared for and evaluated to find those few offspring on which the future industry will be based. In preparation for the pollination period, a plethora of possible pairwise parental permutations are pondered. The breeder must decide the set of cross combinations to make and the target number of seed for each. While the best offspring should carry the best attributes of their two parents, most will probably be only as good as one parent or the other, or combine some or all of the worst traits of each! The fewer redeeming features a potential parent has, and the harder it is to pass those features to the next generation, the poorer its value as a parent and the larger a population must be for a decent chance of spotting a rare elite seedling.

Breeders thus often talk of “breeding value” when considering the suitability of parenthood for individual varieties (cultivars, advanced selections, specialized breeding lines, or germplasm collection accessions). If a variety has many redeeming features that its offspring readily inherit, it is said to have a good “general combining ability”, which follows the same concept as breeding value. In other cases, certain parents may shine only when crossed with certain others, and are then said to have good “specific combining ability”, at least for those specific cross combinations. Breeding values and combining abilities for multiple or single traits can be objectively calculated with well designed crossing experiments, about a decade of dedicated trait evaluation, and advanced training in quantitative genetics. Instead, most Rosaceae breeders have relied on their own experience and a honed breeding instinct – more subjective but nevertheless effective, judging by the extent to which modern cultivars support vibrant industries.

Molecular genetics in the form of *DNA-informed breeding* offers the means of supporting crossing decisions with more direct measures of breeding value than those supplied by quantitative genetics parameters, and provides objective explanations to breeders’ gut feelings. How? Heritable traits are controlled by genes and genetic tests can be developed that monitor the presence and dosage of alleles contributing to the trait. Use of the tests for any age of any plant, from world-famous cultivar to new seedling, will predict what functional alleles the plant carries. This gives a direct window onto its genetic potential for performance and its breeding value. Kate Evans, apple breeder at Washington State University in Wenatchee and RosBREED Demonstration Breeder, is a DNA-informed breeder. For several years, Kate has used DNA-based knowledge of fruit quality retention (at least for firmness) to inform her crossing decisions (Figure 1, next page). The DNA information is not the only factor that Kate takes into account in her crossing decisions, but does assist her decisions – thus the term “marker-assisted”.

Marker-assisted parent selection requires at least one genetic test that a breeder can trust. To date, only a few tests have been available to Rosaceae breeders, such as those highlighted in Amy Iezzoni’s ongoing

continued on page 4

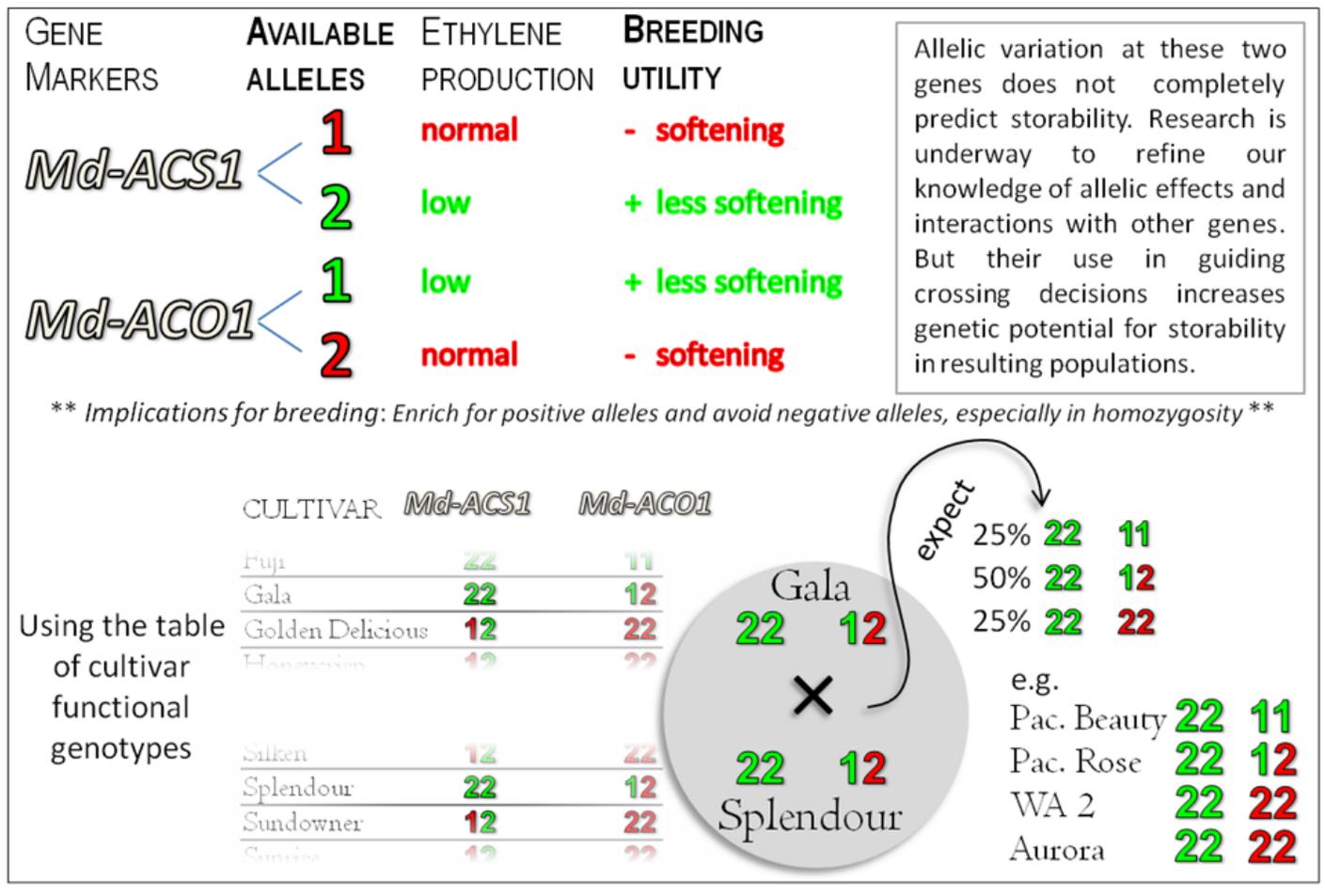
Community Breeders' Page cont.

Jewels in the Genome Newsletter articles. To dispel a myth, there is no need to wait until many predictive tests are available for all the traits a breeder is considering, nor until the genetic tests are 100% predictive of individual traits. DNA information *adds to*, rather than replaces, other information sources considered for crossing, such as each potential parent's performance in field trials and its tendency to pass on useful traits from past crossing experience.

Each genetic test provides useful information that adds to breeders' decision factors at little or no cost to breeders. The predictive genotypes of prospective parents are determined by publicly funded researchers and the knowledge is placed in the public domain. Still, such knowledge can be scattered and garbled in scientific publications that were not written with immediate use by breeders in mind. On the RosBREED Community Breeders webpage, we will compile this rapidly accumulating public domain knowledge on the functional alleles carried by varieties and translate it into forms that you can use. If you would like to gain the genotypic information about your own program's prospective parents, we will also describe the steps for you to obtain it so that the information remains confidential with you.

In coming articles we will describe other examples of Rosaceae breeders using DNA information to help support their critical crossing decisions. What we describe here is just the beginning of a revolution. RosBREED is poised to stock a huge warehouse of predictive genetic tests for you to choose from and enhance your crossing decisions. We will detail the successes and learning experiences of Demonstration Breeders as they shop from this warehouse, while providing the same knowledge accessibility and tools to all of you for con-

Figure 1. Marker-assisted parent selection in apple using gene markers for apple fruit storability. A table of functional genotypes for various apple cultivars can be found at www.rosbreed.org/breeding/jewel-use/



Paternity testing and delivering trait-predictive genotypic data

By RosBREED's Genotyping Team (Nahla Bassil, Barbara Gilmore, and Cameron Peace) assisted by April Nyberg (USDA-ARS-NCGR), Caprice Rosato (OSU), and Bassil Lab members, in collaboration with Demonstration Breeders and Breeding Trainees.

Nahla Bassil sorting apple leaves that were sent to her lab for DNA extraction



Nahla Bassil and Barbara Gilmore extracting DNA from apple leaves



Ksenija Gasic and Terrence Frett (Clemson University) reviewing data sent by Nahla



The Genotyping Team leads the effort to obtain DNA marker genotypes needed to enable marker-assisted breeding for critical fruit quality traits. Genotyping is performed on reference germplasm sets of apple, peach, cherry and strawberry (Figure 1, next page) carefully chosen to represent important breeding parents. In 2010, breeders sent leaves of each chosen tree to the Genotyping Team for subsequent DNA extraction. This effort has resulted in a repository of DNAs needed for genotyping during the RosBREED project.

Over the past few months, these apple, peach, and cherry DNAs were screened with promising trait-predictive DNA markers (Table 1, next page). These markers for high priority fruit quality traits were available yet not fully validated nor widely implemented in Rosaceae fruit breeding programs. The first demonstrations of marker-assisted breeding as facilitated by RosBREED will be conducted with these promising markers in 2011 (while in the meantime huge research efforts in other project areas are expected to provide many further diagnostic tools for fruit quality). Future Newsletters will document advances in breeding efficiency using this marker data. For example, allelic variants of a peach *endoPG* gene should be able to predict whether a peach will have fruit flesh that is rapid-softening, slow-softening, non-softening, free from the stone, or clinging to the stone (described in May 2010's [Jewels in the Genome](#) article). In cherry, a genetic test is available that is expected to simultaneously predict fruit size, firmness, and flavor ([Jewels in the Genome](#), August 2010).

The genotypic data was delivered to the Pedigree-Based Analysis Team to soon validate and assess utility of these markers in specific breeding programs. A very excited group of RosBREED breeders are anxious to apply predictive markers in their programs, starting with refining their crossing decisions by using such knowledge as which of their potential breeding parents are predicted to produce fruit with superior flavor, texture, and size. Also of interest to breeders, early perusal of the genotypic data revealed that some pedigree records are incorrect. Did an individual result from stray pollen? Was there an error in labeling or record-keeping? Such knowledge allows breeders to confirm or reassess their records, and a chance to refine RosBREED's reference germplasm sets for maximized statistical power. While the Pedigree-Based Analysis approach requires pedigree information to be correct to arrive at valid conclusions, so does any study of inheritance and assessment of parental breeding value. This paternity testing is another example of how accessibility to the new tool of DNA-based markers can provide breeders with valuable biological insights.

Paternity testing and delivering trait-predictive genotypic data cont.

Table 1. Genetic tests conducted by the Genotyping Team on crop reference germplasm in early 2011

| Crop | Trait | Locus | Marker |
|--------|--|---|----------------------------------|
| Peach | Fruit flesh softening type (melting, non-melting, non-softening) and flesh adhesion to stone (freestone/clingstone). Also mealiness, firmness, flesh bleeding in storage | <i>F-M</i> locus on <i>Prunus</i> chromosome 4 | SSR: endoPG-1 and SCAR: endoPG-6 |
| Peach | Fruit sweetness (SSC) and acidity (TA) | QTL on <i>Prunus</i> chromosome 4 | SSR: BPPCT023 |
| Apple | Fruit acidity, crispness, and juiciness | <i>Ma</i> locus with QTLs on <i>Malus</i> chromosome 16 | SSRs: CH05c06 and Hi04e04 |
| Apple | Fruit firmness | QTL on <i>Malus</i> chromosome 1 | SSR: Md-Exp7 |
| Cherry | Fruit size, firmness, and flavor | QTL on <i>Prunus</i> chromosome 2 | SSRs: CPSCT038 and BPCT034 |
| Cherry | Fruit acidity | QTL on <i>Prunus</i> chromosome 3 | SSR: BPPCT039 |

Who's Who in the Genotyping Team

Nahla Bassil, Team Leader, USDA-ARS-NCGR



Barbara Gilmore, Research Assistant, USDA-ARS-NCGR



April Nyberg, Research Assistant, USDA-ARS-NCGR



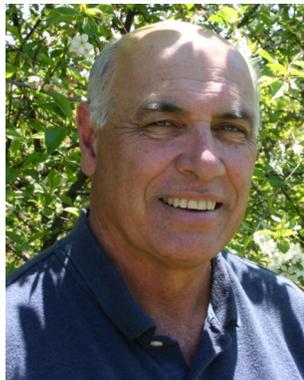
Cameron Peace, Team Advisor, Washington State University



Advisory Panel Member Profile Page

The success of RosBREED will be determined in large part by the people behind it. Because of this, we wanted to give you some insight into these individuals — whether they represent university extension, the scientific community, or industry — who are at the core of Rosaceae. Panel members were asked about their background and what they want to accomplish as part of the RosBREED project. Here is what they had to say.

Extension



RON PERRY

Professor of Horticulture, Dept. of Hort, Michigan State Univ., E. Lansing, Michigan, perry@msu.edu

What work do you do? Initially, my work focused on applied research and extension in the area of rootstocks, high-density systems and root systems/soil relations of tree fruit, including investigations into determining the cause of tart cherry tree decline in Michigan. I also provided to growers results of rootstock field studies of peach, cherry, plum and apple. Additionally, I conducted multi-factorial trials in apple regarding training systems and spacing and extended results to fruit growers through workshops, trade articles, scientific reports, and papers. In recent years, my research and extension interests have moved to orchard and vineyard technology and teaching.

Why are you interested in RosBREED? As a former breeder technician in peach, I found the process in relying on traditional breeding methods to be slow and cumbersome. Our goal was to release a new peach variety within 15 to 20 years after making an initial cross, with a ratio of one commercial variety release in 50,000 seedlings produced and evaluated. I also felt that much improvement could have been made in breeding programs by monitoring industry needs and trends, especially on a global scale. I'm

excited on how RosBREED obtains excellent input from geneticists and genomists around the world to develop genetic markers that can lead to more efficient breeding and selection processes. I also see this project as making a tremendous impact on improving breeder and industrial communications on a local and an international scale.

How do you feel you can contribute to RosBREED? I feel that I can contribute to RosBREED as an experienced pomologist in giving insight into growing and management issues to scientists.

Industry



ROBERT CURTIS

Associate Director, Agricultural Affairs, Almond Board of California, rcurtis@almondboard.com

What work do you do? I administer the Almond Board of California (ABC) production research program. The ABC funds research in horticulture, entomology, plant pathology and nematology, aflatoxin field studies, integrated pest management and pollination. I work to assure field implementation of research findings and hence I am involved in issues which impact almond production and technology transfer. As such, I interact closely with the University of California and other universities, USDA and Cooperative Extension, agriculture organizations and state agencies.

Why are you interested in RosBREED? The RosBREED Project is already contributing to current almond breeding efforts. Improved plant materials is a top priority for the almond industry and ABC-funded programs include variety and rootstock development and evaluation. In variety development, marker-assisted selection has been used to transfer self-fertility from peach to almond. Breeding efforts underway with rootstocks will be assisted by modern genomic tools as well.

How do you feel you can contribute to RosBREED? I feel I can bring the perspective of a stakeholder actively engaged and involved in marker-assisted breeding efforts. The keys to success are breeding pipeline priorities and programs that have commercial application and can be extended to and implemented by industry.

Scientific



PERE ARÚS

Researcher and Scientific Director of Institute for Agricultural and Food Research and Technology (IRTA), Barcelona, Spain, pere.arus@irta.cat

What work do you do? Genetics and genomics of horticultural crops, mainly *Prunus* and to a lesser extent strawberry and melon. Development and use of molecular tools for variability analysis, genetic dissection and understanding of agricultural characters, and applications in commercial breeding of these three species.

Why are you interested in RosBREED? The focus of the project coincides exactly with that of my program in IRTA. The information released by RosBREED is of immediate interest and use in our research. It is also an excellent opportunity to continue and enhance the collaboration with my U.S. colleagues and to explore jointly new ventures and scientific areas.

How do you feel you can contribute to RosBREED? I would like to provide my expertise on the genetics of *Prunus* and strawberry to the project and to share common resources on markers, sequences, materials and ideas on appropriate strategies for application. The recent funding of the FruitBreedomics project by the EU is also an opportunity for me to help in the connection and alignment of these two projects that have similar and complementary goals.

Breeder profile: Susan Brown

By Audrey M. Sebolt, Project Assistant



Box A: Cultivars released in 2010 by Susan Brown, Cornell University, New York



NY 1: This hybrid of 'Honeycrisp' by an advanced breeding selection, has the desirable attributes of 'Honeycrisp', without its production challenges. This fresh market cultivar is crisp, juicy and sweet. Trees are not susceptible to powdery mildew, bitter pit, leaf chlorosis or fireblight. NY 1 is also annual bearing.



NY 2: This variety has a wonderful blend of sweetness and tartness, along with exceptional crisp, firm fruits that store well. Fruits are suitable for both the fresh and processing markets and have high vitamin C content.

MDT-1 measures the crispness and firmness of apples.
Photo: Kate Evans, Wash. State Univ.



Dr. Susan Brown began breeding apples at Cornell University in 1990. Each year her program harvests 7,000 to 10,000 seeds from crosses. Her program manages 73 acres that include clonal trials of advanced selections, named cultivars and seedlings. Her primary focus is developing and releasing new cultivars for the fresh apple market. The Cornell University breeding program has released 66 apple cultivars, including 'Empire', 'Macoun', 'Jonagold', and 'Cortland'. In 2010, Susan released two advanced selections, New York 1 and New York 2 (NY 1 and NY 2 respectively; soon to be given trademarked names) in a cooperative partnership with NYAG, LLC, a cooperative open to all apple growers in New York State (Box A). NY 1 took only 12 years from cross to release because Susan saw the potential of this cultivar when it first fruited. The following spring, her program propagated trees of NY 1 for Cornell and grower trials. NY 2 took 18 years from cross to release. By 2013, it is estimated that there will be 900 acres of these two varieties planted in New York orchards.

Susan seeks germplasm for use in crosses for new and improved cultivars that have high vitamin C levels, unique and distinctive attributes such as color that "feathers" across the surface, outstanding quality including aroma, crispness, juiciness, firmness and a balance of high sweetness and high acidity that is maintained while in storage. An example of a cross that Susan made is 'Autumn Crisp' by 'Braeburn'. 'Autumn Crisp' was chosen because it has low browning, which is ideal for the consumer and the apple slice market, and high vitamin C content. However, this variety does not hold up well in storage. 'Braeburn', which has even higher vitamin C content, has good storage life, so this cross should have good vitamin C content and a diverse range of storage abilities. Seedlings from this cross are evaluated once they fruit, however, their overall "package" of a tree that will maximize grower profits and a fruit that will be accepted by the fresh market must meet and exceed the traits of other cultivars already on the market.

Susan is excited about the opportunity, through RosBREED, to collaborate with other apple and Rosaceae breeders in the United States. Standardizing phenotyping protocols within crop teams allows programs to compare data using the same metrics. In the past, crispness and firmness of apple selections was tested using a penetrometer or by biting into an apple. Generating data for this trait was time consuming and often subjective, depending on the tester. Jim Luby (University of Minnesota), Kate Evans (Washington State University), and Susan each decided to purchase an MDT-1 penetrometer (pictured left), developed by Mohr and Associates. This machine enables these three apple programs to more accurately

continued on page 9

Breeder profile continued**Traits Susan is breeding for:**

- Distinctive and consistent fruit quality: flavor, firmness, crispness, and juiciness
- Fruit flavor including enhanced sweetness and acidity
- Reduced flesh browning – for the fresh market and the apple slice market
- Storage and shelf life, with freedom from storage disorders
- Higher antioxidant content (including vitamin C)
- Distinctive fruit appearance: skin color, hue, and stripes
- Disease resistance: scab and fireblight

and quickly measure the crispness and firmness of apples and can provide measurements for over 1,000 samples per day.

While marker-assisted breeding is a long term goal, Susan has been frustrated by a lack of reliable markers for traits of commercial importance. Susan feels that the DNA diagnostic markers that are developed through RosBREED efforts will be very powerful because they will be tested in three environments, Minnesota, Washington, and New York, and on a wide array of different genetic backgrounds.

Enhanced communications among Demonstration Breeders and interactions with the supporting research community are also benefits that Susan feels are important. She has attended both Annual RosBREED Advisory Panel Member meetings and values the interactions and feedback offered by Industry, Scientific, and Extension specialists.

Meet Susan's Breeding Trainee: Benjamin Orcheski

Why did you choose Susan Brown's program? I am currently working for my PhD in Horticulture in Susan Brown's lab. For years I have had an interest in the genetics of fruit crops (marvels of evolution). I discovered Susan through her website and was both fascinated and impressed by the work she is doing. After meeting with her and finding out first hand what a knowledgeable and fun person she is, I knew Cornell was the natural choice for me.

What is your thesis project if it has been determined? My project is based on studying epigenetic changes (DNA methylation pattern) in apple interspecific hybrids, and their relation to altered tree architecture and morphology.

What benefits have you seen to being part of RosBREED? I see two great benefits from being part of the RosBREED project. The first is learning about the theory and practice behind QTL mapping. I admittedly

knew very little about this facet of genetics before joining the project and am still learning a great deal. I have also gained an appreciation for the challenges and goals that each breeder has for their particular species (especially apple). It's really fascinating to hear each breeder's opinion of what the idealized fruit or plant would look like and how they want to achieve that.

The second benefit from being part of RosBREED is the friendships and connections that get built as the work progresses. It is really nice to work with like-minded individuals who have a common goal and appreciation. Also, understanding the mechanics and organization of a large scientific endeavor has been very helpful to me as a student and aspiring scientist.

Jewels in the genome

By Amy Iezzoni, Project Director

What is a "Jewel in the Genome?"

- *An individual's genome is the full complement of genetic information that it inherited from its parents. Within this vast repertoire of genetic information, individual genes are being discovered that control critical production and fruit quality traits. As these valuable rosaceous gene discoveries are made and put into breeding applications, we will describe them in this column as "Jewels in the Genome."*

Skin and flesh color in apple and cherry fruit varies widely from dark red to almost colorless due to differences in anthocyanin pigmentation. This fruit skin and flesh color variation is used to define market types with individual consumers, while food manufacturers frequently have a color preference. In apple (and many other fruits), members of an anthocyanin-activating group of genes called MYB transcription factors control this variation for red color. In apple, one particular member of the *MYB* gene family controls both red skin and flesh color.

Certain alleles for this *MYB* gene results in exciting flesh colors that are being selected by breeders. Apple cultivars are in development that have completely red flesh and others have flesh with lesser amounts of red color in interesting patterns. These new fruit types, touted due to reported health benefits of anthocyanins, should be available for consumers in just a few years.

Because of the shared ancestry of rosaceous crops, it was hypothesized that the same *MYB* gene may control skin and fruit color in cherry. This turned out to be the case and led to the relatively quick discovery that *MYB* gene variation is primarily responsible for major color differences among sweet cherry market classes, such as the dark mahogany flesh and skin of 'Bing' cherries compared with the light yellow-fleshed and slightly red-blush of 'Rainier' cherries. Scientists are exploring the hypothesis that the same gene also influences fruit color in other rosaceous fruit such as peach and strawberry

With genetic knowledge of *MYB* variants in their plant material, breeders can harness this wide array of genetic diversity to make desirable parental combinations and select seedlings with superior traits prior to field planting. In some cases, such as red apple flesh color, these new varieties will have exciting new appearances not widely available previously. Therefore, because this *MYB* gene will lead to more efficient breeding of apples and cherries with desirable appearance and higher nutraceutical levels, it is chosen as our fifth featured "Jewel in the Genome."



Photo: USDA-ARS

Bing sweet cherry



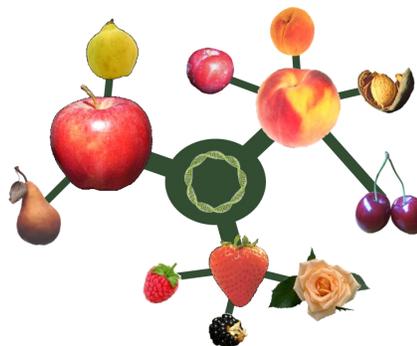
Rainier sweet cherry



RosBREED: Enabling marker-assisted breeding in Rosaceae

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| Cholani Weebadde | Extension | Michigan State University |
| Chengyan Yue | Socio-economics | University of Minnesota |

Calendar of events

- **March 8-11, 2011: RosBREED General Meeting and Workshop, East Lansing, MI.**
- **September 25-28, 2011: American Society for Horticultural Sciences will meet in Hawaii. For more details, please visit [ASHS](http://www.ashs.org).**